RESEARCH ARTICLE

Prevalence and Co-infection of Intestinal Parasites among Thai Rural Residents at High-risk of Developing Cholangiocarcinoma: A Cross-sectional Study in a Prospective Cohort Study

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Abstract

Intestinal parasitic infections (IPIs) are still important to the health of Thai rural residents. IPIs are the cause of many chronic diseases with, for example, opisthorchiasis resulting in progression to cholangiocarcinoma (CCA). This cross-sectional study in a prospective cohort study aimed to examine the prevalence and co-infection of intestinal parasites among Northeastern Thai rural residents, recruited into the Khon Kaen Cohort Study (KKCS), and who were residing in areas of high-risk for developing CCA. On recruitment, subjects had completed questionnaires and provided fecal samples for IPI testing using the formalin ethyl acetate concentration technique. Data on selected general characteristics and the results of the fecal tests were analysed. IPI test results were available for 18,900 of cohort subjects, and 38.50% were found to be positive for one or more types of intestinal parasite. The prevalence of *Opisthorchis viverrini* (*O. viverrini*) infection was the highest (45.7%), followed by intestinal flukes (31.9%), intestinal nematodes (17.7%), intestinal protozoa (3.02%), and intestinal cestodes (1.69%). The pattern of different infections was similar in all age groups. According to a mapping analysis, a higher CCA burden was correlated with a higher prevalence of *O. viverrini* and intestinal flukes and a greater intensity of *O. viverrini*. Both prevention and control programs against liver fluke and other intestinal parasites are needed and should be delivered simultaneously. We can anticipate that the design of future control and prevention programmes will accommodate a more community-orientated and participatory approach.

Keywords: Prevalence - co-infection - intestinal parasites - cholangiocarcinoma - Thailand

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Introduction

Intestinal parasitic infections (IPIs) are globally endemic and have been described as constituting the greatest single worldwide cause of illness and disease (Curtale et al., 1998; Steketee, 2003). The high prevalence of these infections is closely correlated with lack of sanitation, lack of access to safe water, improper food preparation, improper hygiene and impoverished health services (Albonico et al., 1993). Several studies have reported that the people most infected with intestinal and liver parasites are rural residents, especially those in developing countries in, for example, Africa (Koukounari et al., 2008; Nyarango et al., 2008), Asia (Harinasuta et al., 1976; Sithithaworn et al., 2006; Mehraj et al., 2008; Sayasone et al., 2009), and South America (Carme et al., 2002; Carvalho-Costa et al., 2007).

The northeast region of Thailand is a mostly dry geographical area and, in socioeconomic terms, has been the most impoverished part of the country for decades. The region contains about a third of the population of Thailand, and most of these people live in largely rural environments where their lifestyle exposes them to the risk of parasitic infection. Intestinal parasitic infections, including protozoal infections, remain a major threat to their health. These infections cause chronic conditions, which may progress to serious diseases. This is especially true of opisthorchiasis, which is due to infection by *Opisthorchis viverrini (O. viverrini)*, and can result in cholangiocarcinoma (CCA), the commonest form of cancer in the northeast region (Vatanasapt et al., 1990; Parkin et al., 1993; Sriamporn et al., 2004; Sripa et al., 2007).

The aim of this study was to report on the infection

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and co-infection of IPIs in a population of Khon Kaen Province, which had been recruited for the Khon Kaen Cohort Study (KKCS) (Sriamporn et al., 2005), and report on the distribution by age and sex of those with the various IPIs.

This research is part of the project, "A prospective study of cancer and other outcomes in a rural population in Thailand", which was approved by the Khon Kaen University Ethics Committee for Human Research in accordance with the Declaration of Helsinki and the Good Clinical Practice Guidelines (ICH GCP), Reference No. HE512053.

Materials and Methods

Data collection

During 1990-2001, the phase of recruitment to the study cohort, villages in Khon Kaen Province were selected for participation, and the eligible population (30-69 years) recruited during 2-3 weeks of fieldwork. All 25 districts were included. Initially, one sub-district in each district was selected at random, and one of the villages in the sub-district was chosen on the recommendation of the local health personnel to maximize compliance. Later, for some districts, screening took place in all sub-districts, and, in other districts, all of the villages in the selected sub-district were screened. For some districts, more than one sub-district was sampled. As a result, the sampling fraction by district and the time period during which the populations of different districts were tested were quite variable.

A fecal sample was collected by all participants in a small plastic container marked with their name and identification number. The samples were stored in an ice-box for 2-3 days before being transferred to the parasitological laboratory at the Faculty of Medicine, Khon Kaen University. The samples were examined within 24 hours after arrival by the quantitative formalin ethyl acetate concentration technique (Elkins et al., 1990; Intapan et al., 2005). Briefly, two grams of each fecal sample were weighed out and then stirred well in a vial containing a merthiolate-iodine-formalin solution. The preserved fecal suspension was filtered through two layers of wet gauze into the centrifuge tube. The volume was adjusted to 10 milliliters with 10% formalin. Two milliliters of ethyl acetate were added, and the tube was closed and shaken for half a second. The stopper was removed, and the tube was centrifuged at 600x g for 10 minutes. The plug of debris along with the merthiolate-iodine-formalin solution was discarded by inverting the tube, leaving only the sediment, which was re-suspended. The entire suspension was examined under a light microscope. Intestinal parasites eggs in the sediment were counted and recorded separately for each species.

After the fecal examination, the Stoll egg counting technique was applied to specimens which were positive for the eggs of *O. viverrini* (Kurathong et al., 1984). Based on the eggs per gram of feces (EPG), the specimens were classified as Grade I (EPG<500), Grade II (EPG 500-1,799), Grade III (EPG 1,800-9,999) and Grade IV (EPG over 10,000) as described in previous reports (Haswell-Elkins et al., 1994).

Data analysis

Descriptive statistics were used to summarize general characteristics, including the presence of IPIs. Frequencies and percentages were used to present the categorical data, while means and standard deviations (SDs) were used for continuous data. The percent (%) of intestinal parasitic-infected individuals in each district was stratified by sex, age at recruitment, stool examination, parasite eggs in stool, type of parasitic infections and multiple infections of intestinal parasites of the district population.

Mapping analysis was used to show the prevalence of IPIs and intensity of *O. viverrini* among rural residents at high-risk of developing CCA according to each district of Khon Kaen Province. The class intervals adopted for CCA incidence were <109, 110-169, 170-229, 230-279, >280 per 100,000 person years as previously described (Sriamporn et al., 2004; Sripa et al., 2007).

Results

The records of 18,900 individuals aged 30-69 years, whose stool samples had been examined, were included in the analysis. Four age groups were created (30-39, 40-49, 50-59 and 60-69 years). The presence of IPIs was analyzed according to five parasite groups: (1) intestinal protozoa, comprising of *Entamoeba coli (E. coli)*, *Entamoeba histolytica (E. histolytica)* and *Giardia lamblia (G. lamblia)*, (2) intestinal flukes, comprising of *Echinostoma* spp. and minute intestinal flukes, (3) liver fluke, comprising of *O. viverrini*, (4) intestinal cestodes, comprising *Hymenolepis diminuta (H. diminuta)*, *Hymenolepis nana (H. nana)*, and *Taenia* spp. and (5) intestinal nematodes, comprising of *Ascaris lumbricoides (A. lumbricoides)*, Hookworms, *S. stercoralis* and *Trichuris trichiura (T. trichiura)*.

Table 1 shows the distribution of sex, age at recruitment and results of stool examination of study subjects. There were 38.5% positive for any kinds of intestinal parasites. The total number of parasite infections (including single and multiple infections) in the 7,276 subjects with at least one type of infection was 9,697 (3,690 in males and 6,007 in females).

The details of the presence of IPIs among rural residents stratified by sex are presented in Table 2. The presence of *O. viverrini* infection was 45.69%, and the gender difference was very small. The presence of intestinal flukes was 31.89%: 16.10% were minute intestinal flukes, for which the prevalence was higher in females (18.86%) than in males (11.60%), while 15.79% were *Echinostoma* spp. for which the prevalence was lower in females (13.17%) than in males (20.05%). The prevalence of intestinal nematodes was 17.71%, and infection by Hookworms accounted for highest prevalence (14.45%) in this group of IPIs. Most subjects with Hookworms infection were female. The prevalences of intestinal protozoa and cestodes were 3.02% and 1.69%, respectively.

The prevalence of intestinal parasitic-infected individuals by age at recruitment is shown in Figure 1. All age groups showed the same pattern: liver fluke infection had the highest prevalence, followed by infections of intestinal flukes, intestinal nematodes, intestinal protozoa, and intestinal cestodes.

Figure 2 shows the prevalence of intestinal parasitic infections, intensity of *O. viverrini* infection and risk of developing CCA among rural residents in each of the 25 districts of Khon Kaen Province. The increasing intensity of red represents increasing incidence of CCA, while the increasing number of asterisks represents the increasing intensity of *O. viverrini* infection based on the EPG grade as described above (Haswell-Elkins et al., 1994). The prevalence of IPIs was shown for: a. intestinal protozoa, b. intestinal flukes, c. liver fluke (*O. viverrini*), d. intestinal cestodes and e. intestinal nematodes, respectively. Data for the class intervals adopted for CCA incidence were available from previous studies (Sriamporn et al., 2004; Sripa et al., 2007). The highest incidences of CCA (>280 per 100,000 person years) were in Nong Na Kham, Phu Wiang, Mancha Khiri, Khok Pho Chai, Chonnabot, Non Sila and Pueai Noi (red districts on map). Most of these districts also had higher prevalences of infections by *O. viverrini* and intestinal flukes than the other districts (16.94% of *O. viverrini* infection and 10.26% of intestinal fluke infection in Chonnabot, for example). The intensities of *O. viverrini* infections in the red districts were Grade II or III (mean scores between 500-1,799 and 1,800-9,999 EPG). Nong Ruea was the only district in the second class interval (230-279 per 100,000 person years and orange district on map). It is notable that the intensity of *O. viverrini* infection in this area was Grade IV (EPG over 10,000), which is the highest grade of intensity of *O. viverrini* infection. Districts in the third class interval (170-229 per 100,000 person years) were Si Chomphu, Kranuan, Sam Sung, Phrayuen, Ban Haet, Ban Phai, Waeng Noi and Waeng Yai (green districts on map), and the intensities of *O. viverrini* infection in these districts were less than 500 EPG.

In collaboration with the local primary health care centers, all participants, whose fecal sample was found to be positive for one or more type of intestinal parasite eggs, were informed of their results and advised to seek treatment. Each case was treated individually, according to the type of infection. For example, a single dose of 500 mg mebendazole was prescribed for helminths, except for *Strongyloides stercoralis* (*S. stercoralis*), for which a dose of 400 mg albendazole was given for three consecutive days. Metronidazole (15 mg/kg/day for 7 days) was administered for protozoan infections, and cases with *O. viverrini* eggs were treated with 75 mg/kg praziquantel, administered as three 25 mg/kg doses on a single day.

Table 1. The Distribution of Sex, Age, Results of Stool Examination and Multiple Infections of Intestinal Parasites among Participants in Khon Kaen, Thailand

Variables	Male No.	Male %	Female No.	Female %	Total No.	Total %
Sex	6,162	32.6	12,738	67.4	18,900	100
Age at recruitment (years)						
30-39	558	9.06	1,811	14.22	2,369	12.53
40-49	2,042	33.14	4,462	35.03	6,504	34.41
50-59	2,344	38.04	4,447	34.91	6,791	35.93
60-69	1,218	19.76	2,018	15.84	3,236	17.12
Mean ± SD	50.9±8.7		49.8±8.9		50.2±8.8	
Median (min: max)	51 (30:69)		50 (30:69)		50 (30:69)	
Parasite eggs in stool[a,b]						
Absence	3,444	55.89	8,180	64.21	11,624	61.5
Presence	2,718	44.11	4,558	35.79	7,276	38.5
Total	6,162	100	12,738	100	18,900	100
Multiple infections of intestinal parasites in infected group						
Single infection	1,923	70.75	3,349	73.48	5,272	72.46
Double infections	641	23.58	995	21.83	1,636	22.48
Triple infections	132	4.86	190	4.17	322	4.43
Tetra infections	21	0.77	22	0.48	43	0.59
Penta infections	1	0.04	2	0.04	3	0.04
Total	2,718	100	4,558	100	7,276	100

[a]Stool examinations were detected by formalin ethyl acetate concentration technique; [b]Parasite eggs in stool were quantitative recorded as absence or presence of any type of parasite

Table 2. Types of Intestinal Parasitic Infections of Participants in Khon Kaen, Thailand, by Number of Parasites

Intestinal parasitic infections	Male No.	Male %	Female No.	Female %	Total No.	Total %
Intestinal Protozoa						
Entamoeba coli	23	0.62	116	1.93	139	1.43
Entamoeba histolytica	1	0.03	2	0.03	3	0.03
Giardia lamblia	62	1.68	89	1.48	151	1.56
Intestinal flukes						
Echinostoma spp.	740	20.05	791	13.17	1,531	15.79
Minute intestinal flukes	428	11.60	1,133	18.86	1,561	16.10
Liver fluke						
Opisthorchis viverrini	1,722	46.67	2,709	45.10	4,431	45.69
Intestinal cestodes						
Hymenolepis diminuta	1	0.03	1	0.02	2	0.02
Hymenolepis nana	0	0	1	0.02	1	0.01
Taenia spp.	82	2.22	80	1.33	162	1.66
Intestinal nematodes						
Ascaris lumbricoides	8	0.22	9	0.15	17	0.18
Hookworms	436	11.82	965	16.06	1,401	14.45
Strongyloides stercoralis	177	4.80	104	1.73	281	2.90
Trichuris trichiura	10	0.27	7	0.12	17	0.18
Total	3,690[a]	100	6,007[a]	100	9,697[a]	100

[a]Number of parasites

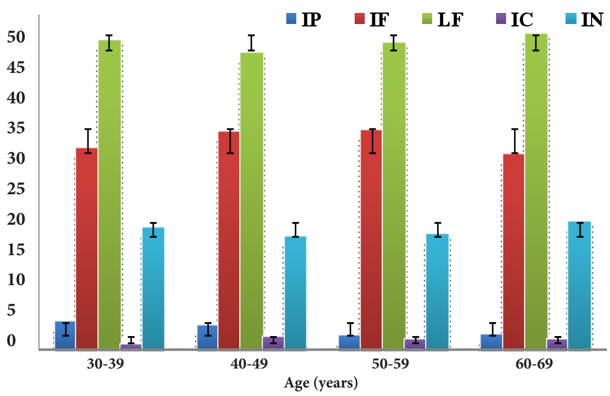

Figure 1. Prevalence of Intestinal Parasitic Infections of Residents in Khon Kaen, by Age at Recruitment. IP, Intestinal protozoa (*Entamoeba coli, Entamoeba histolytica* and *Giardia lamblia*); IF, Intestinal flukes (*Echinostoma* spp. and Minute intestinal flukes); LF, Liver fluke (*Opisthorchis viverrini*); IC, Intestinal cestodes (*Hymenolepis diminuta, Hymenolepis nana* and *Taenia* spp.); IN, Intestinal nematodes (*Ascaris lumbricoides*, Hookworms, *Strongyloides stercoralis* and *Trichuris trichiura*)

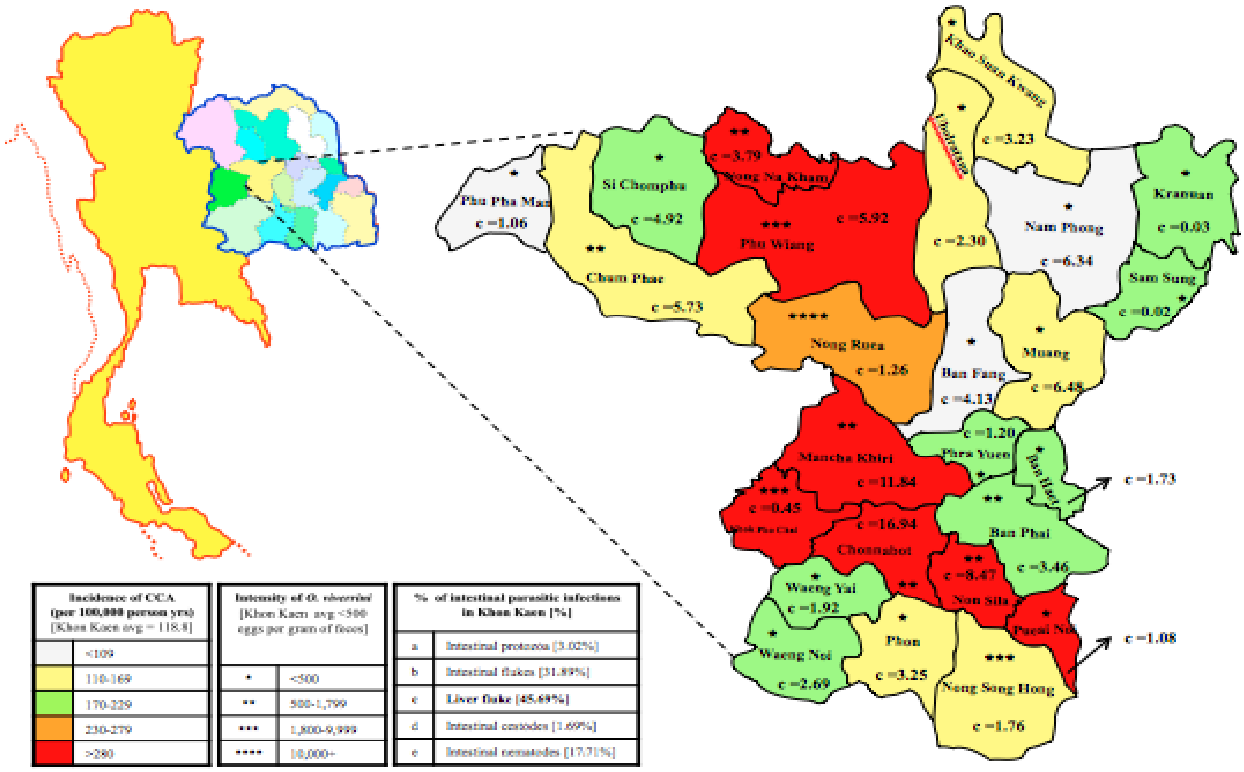

Figure 2. Prevalence of Intestinal Parasitic Infections and Intensity of *Opisthorchis viverrini* of Residents at High-risk of Developing Cholangiocarcinoma, by Districts of Khon Kaen. The class intervals adopted for CCA incidence were used as previously described (Sriamporn et al., 2004; Sripa et al., 2007)

Discussion

This was a cross-sectional study describing the percentage of prevalence and co-infection of intestinal parasites among Thai rural residents, who were living in areas associated with a high-risk of developing CCA (Vatanasapt et al., 1990; Parkin et al., 1993; Sriamporn et al., 2004; Sripa et al., 2007). Results of faecal examinations conducted with the formalin ethyl acetate concentration technique.

38.50% of these were positive for one or more types of intestinal parasite. *O. viverrini* infection was found to have the highest prevalence (45.69%), followed by intestinal flukes (31.89%), intestinal nematodes (17.71%), intestinal protozoa (3.02%), and intestinal cestodes (1.69%). Importantly, the pattern of the different infections was similar in all age groups. It is notable that the two most common IPIs endemic in the study cohort were infections by liver and intestinal flukes and that these parasites share the same intermediate host (cyprinoid fish). In the past, many people preferred classical culinary dishes, which involved the use of uncooked cyprinoid fish (especially *Koi-Pla*) (Parkin et al., 1991; Srivatanakul et al., 1991; Sriamporn et al., 2004; Sripa et al., 2007), and the ingestion of dishes made with uncooked or undercooked fish continues to occur (Sithithaworn et al., 2012). Although there have been several preventative programmes designed to change the practice of eating uncooked or undercooked cyprinoid fish, this age-old, culturally-embedded culinary habit still seems to persist.

According to the mapping analysis, the areas shaded in red represent districts where the incidence of CCA is greater than 280 per 100,000 person years. These areas are associated with expanses of natural water, which are productive fishing resources; for example, a major watercourse (the Chi River) and its tributaries run through Mancha Khiri, Khok Pho Chai, Chonnabot, Non Sila and Pueai Noi, and Nong Na Kham and Phu Wiang are adjacent to the large Ubolratana multi-purpose reservoir. A higher CCA burden is correlated with a higher prevalence of *O. viverrini* and intestinal flukes and with greater intensity of *O. viverrini* infection, although sporadic anthelmintic therapy has influenced this relationship. It is noted that even one asterisk represents a cancer rate, which would be regarded as high anywhere else in the world (Sripa et al., 2007). For the analysis *O. viverrini* infection in relation to CCA risk, study subjects should be periodically followed-up, perhaps every three years. However, the costs and feasibility of this are important considerations.

From a public health perspective, the proper cooking of cyprinoid fish can efficiently block infection by food-borne intestinal parasites related to CCA in the endemic area. There has been a number of large-scale national control programmes in Thailand (Jongsuksuntigul and Imsomboon, 1998; 2003), and there is a current local project (the "Lawa model programme") in Khon Kaen Province (Sithithaworn et al., 2012), but a high prevalence of *O. viverrini* infections still remains in the endemic areas. The main reason of the failure of the previous control programs is likely to be the lack of culturally sensitive and well-formulated health education about raw food consumption attitudes and practices (Grundy-Warr et al., 2012). Both prevention and control programs against liver flukes and other intestinal parasites are needed and should be delivered simultaneously. Control programs using EcoHealth methodologies, such as the "Lawa model" (Grundy-Warr et al., 2012), represent one of the approaches which may be ideal for opisthorchiasis control. We can anticipate that the design of future control and prevention programmes will accommodate a more community-orientated and participatory approach.

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